

CLAIMS

1. A process for purifying alpha-1 proteinase inhibitor (API) from an unpurified mixture of proteins comprising:
 - a. dispersing the unpurified mixture of proteins containing API in an aqueous medium;
 - b. removing a portion of contaminating lipids and proteins by adding a lipid removal agent to the aqueous dispersion and precipitating the portion of contaminating proteins from said aqueous dispersion;
 - c. loading the API-containing supernatant of step (b) containing API on a first anion exchange resin with a buffer solution having pH and conductivity such that API is retained on the first anion exchange resin;
 - d. eluting an API-containing fraction from said first anion exchange resin with the same type of buffer as in step (c) having adjusted pH and conductivity;
 - e. loading the API-containing fraction of step (d) on a cation exchange resin in said same type of buffer having appropriate pH and conductivity such that API is not retained on the cation exchange resin;
 - f. collecting the flow-through of step (e) that contains API;
 - g. loading the API-containing fraction of step (f) on a second anion exchange resin with said same type of buffer having appropriate pH and conductivity such that API binds to the second anion exchange resin;
 - h. eluting API from said second anion exchange resin with said same type of buffer having adjusted pH and conductivity to obtain a solution containing purified, active API.
2. The process of claim 1, wherein the API obtained comprises at least 90% active API out of the total API recovered.
3. The process of claim 2, wherein the API obtained comprises at least 95% active API out the total API recovered.
4. The process of claim 1, wherein the API solution comprises at least 90% API out of the total protein recovered.
5. The process of claim 4, wherein the API obtained comprises at least 95% API out of the total protein recovered.

6. The process of claim 1, wherein the buffer solution is other than citrate based buffer.
7. The process of claim 1, wherein the buffer solution is acetate-based buffer.
8. The process of claim 1 further comprises a viral inactivation step.
- 5 9. The process of claim 8 wherein the viral inactivation step comprises adding a solvent and a detergent to the API of step (f) collected from the cation exchange resin.
- 10 10. The process of claim 9 wherein the detergent is a non-ionic detergent.
11. The process of claim 1, further comprising a viral removal step.
- 10 12. The process of claim 11, wherein the viral removing step comprises nanofiltration.
13. The process of claim 1, wherein the unpurified mixture of proteins is selected from the group consisting of Cohn Fractions, human blood plasma and plasma fractions.
- 15 14. The process of claim 13 wherein the unpurified mixture of proteins is Cohn fraction IV- paste.
15. The process of claim 1 wherein the lipid removing agent is silicon dioxide.
16. The process of claim 1 wherein the portion of contaminating lipids and proteins is precipitated by polyalkylene glycol.
- 20 17. The process of claim 16, wherein the polyalkylene glycol is polyethylene glycol.
18. The process of claim 16 wherein precipitation is performed at a pH from about 5.0 to about 6.5.
19. The process of claim 1, wherein the first and the second anion exchange resin is a DEAE-Sephadex resin.
- 25 20. The process of claim 1 wherein the cation exchange resin is Carboxymethyl-Sephadex resin.
21. The process of claim 1, wherein the pH of the buffer solution is at a pH of between 5.5 and 6.5 for the elution of the API from the first and the second

anion exchange resin.

22. The process of claim 1, further comprising changing the ionic composition of the solution containing purified, active API to contain a physiologically compatible ion and sterilizing the resulted solution.
- 5 23. The process of claim 22, wherein the solution containing API is concentrated before the ion exchange.
24. The process of claim 22, wherein the physiologically compatible ion is selected from the group consisting of a phosphate ion, a chloride ion and combinations thereof.
- 10 25. A purified active API produced by the process of any one of claims 1-24.
26. A pharmaceutical preparation comprising as an active ingredient a purified active API produced by the process of any one of claims 1-24.
27. A pharmaceutical preparation comprising a purified, active API in a form of a ready to use sterile solution.
- 15 28. The pharmaceutical preparation of claim 27 wherein the pH of the composition is in the range of 6.5-7.5.
29. The pharmaceutical preparation of claim 27 wherein the protein concentration is between about 1% to about 3%.
30. The pharmaceutical preparation of claim 27, wherein the composition is devoid
20 of a protein stabilizer.
31. The pharmaceutical preparation of claim 30, wherein the API is stable for at least 3 months, preferably 4 months, more preferably 6 months when the pharmaceutical preparation is stored in a temperature range of between 20°C to 25°C.
- 25 32. The pharmaceutical preparation of claim 30, wherein the API is stable for at least 12 months, preferably 24 months, more preferably 36 months, when the pharmaceutical composition is stored in a temperature range of between 2°C to 8°C.
33. A pharmaceutical composition comprising as an active ingredient a purified
30 active API produced by the process of any one of claims 1-24, further

comprising an excipient, a diluent or a carrier.

34. The pharmaceutical composition of claim 33, formulated to be administered intravenously.

5 35. The pharmaceutical composition of claim 33, formulated to be administered by inhalation.

36. A method for treating a subject in need thereof comprising administering a therapeutically effective amount of API produced by the process of any one of claims 1-24.

10 37. The method of claim 36 for treating a disease or disorder selected from the group consisting of pulmonary emphysema, chronic obstructive pulmonary disorder, cystic fibrosis associated lung diseases and disorders, psoriasis and atopic dermatitis.

38. The method of claim 37 for treating pulmonary emphysema.

15 39. The method of claim 37 for treating cystic fibrosis associated lung disease or disorder.